**How can nanoparticles help neural cell transplantation therapy?**

Nano-sized particles (size < 500 nm) are a major emergent platform for experimental neurology and cell therapy. Nanoparticle (NP) development in recent years has advanced in parallel with cell transplantation therapies for neurological disease and injury [1]. Consequently, a fusion of NP platforms with neural transplantation can offer a powerful hybrid technology to develop next generation cell therapies.

NPs can be formulated from organic (liposomes, dendrimers and other polymers) or inorganic materials (metals, metal oxides and metalloids) and their chemical structure underpins their versatility for functionalization. The strategic design of inorganic-organic hybrid NPs, in particular, offers high potential for multi-tasking where the fundamental design consists of an inorganic core, with a protective coating which can be decorated with functional organic molecules/bioactive reagents. The core can provide chosen physical characteristics such as magnetism/magnetic resonance (MR) (T2) contrast from iron oxide (e.g. Fe3O4), computerized tomography (CT) contrast from gold (Au), or optical (luminescent) properties from quantum dots (QDs) [2]. Other nanoparticle cores, such as silica and poly(lactic-co-glycolic acid))) can be used as non-functional hosts for active bio-reagents. The coatings protect NP cores from corrosion and elements leaching into the cellular microenvironment. They can also serve as a “linkage” layer for bioactive reagents such as genetic material, drugs, or antibodies [3]. This offers important advantages over conventional ‘single-purpose’ agents for transplantation neurobiology research and clinical use, such as fluorescent dye labelling for cell tracking or virus-based gene transfer for cell engineering.

In terms of neural cell therapy, NPs have found the greatest application so far in medical imaging, to label transplant populations for graft tracking in host neural tissue. This enables correlations to be made between neurological recovery and cell migration, or off target delivery and related safety issues, for example. Superparamagnetic iron oxide nanoparticles or SPIONs (e.g. Fe3O4) have been used to label neural transplant populations for imaging using MRI or magnetic particle imaging (MPI; a new high-resolution tomographic technique) [4]. MRI is widely available in clinical settings and imaging of labelled cell suspensions has been tested for many neural transplant populations (e.g. oligodendrocyte precursor cells/OPCs, mesenchymal stem cells/MSCs, neural stem cells/NSCs, olfactory ensheathing cells/OECs, embryonic stem cells/ESCs, astrocytes, bone marrow and adipose derived stem cells) using MRI. The need for specialist instrumentation, however, is a major consideration for clinical translation of imaging platforms such as MPI. Quantum dots have been explored for neural cell applications but have seen a decline with safety concerns over leaching of toxic elements (e.g. Cd, Te, Se) [5]. Their superior luminescence properties (e.g. anti-bleaching), however, are still unmatched by organic competitors including most fluorophores. Early stage alternatives such as carbon dots or polymer dots (polymeric carbon nanoparticles) display luminescence with lower toxicity and have been trialed for stem cell labelling, but their neural applications are relatively unexplored [6].

Another proven application for NPs in neural cell therapy is in the control of stem cell properties via genetic engineering (with DNA/siRNA) or delivery of small molecules [7], sometimes in combination (for example, retinoic acid and siSox9 to neural stem cells). Positively charged surfaces and functional groups are essential to bind nucleic acids and most coatings for this purpose are amine-type functional groups. Polyethyleneimine (PEI) is popular due to its wide availability, low cost and low toxicity. Low molecular weight and straight chains are less toxic than high molecular weight or branched PEI. Positively charged biopolymers such as chitosan and polylysine and other “designer” polymers have also been used for biomolecule delivery; for example, NPs coated with poly(amidoamine) dendrimers, but the latter involve laborious synthesis and purification processes making scale-up for biomedical applications a challenge [8].

Magnetic cores including SPIONs can allow for transfection grade NPs to be deployed with external static and oscillating magnetic fields in vitro (‘magnetofection’ or magnet assisted transfection technology), a strategy demonstrated to greatly improve transfection outcomes [9]. Oscillating magnetic field devices generally outperform static ones and many variants of magnetic field devices have been tested with variable outcomes; it is currently unclear what device format will yield optimal transfection for neural cells.

Major neural transplant types such as NSCs, OPCs, astrocytes, NSCs and OECs have been proven to be amenable to nano-engineering, including in some cases using genes encoding neurotrophins such as BDNF and FGF2 [9]. DNA size correlates inversely with NP transfection efficiency for reasons that are largely unknown, but the use of new DNA vectors such as DNA minircles can limit this problem and enhance transfection, as can repeated NP application (termed ‘multifection’) [9]. Potentially, functionalizing nanocarriers with a targeting cell penetrating peptide (CPP) could also enhance uptake of transfection grade particles into transplant populations and represents a sophisticated targeting approach, but a lack of cell specific neuro-CPPs is currently a major obstacle to such a delivery strategy.

A significant hurdle for neural cell therapy is low transplant survival and integration after surgical microinjection into the neural parenchyma, probably due to the high mechanical forces and cell clumping experienced by grafted cells during surgical injection. This contributes to inadequate neurological outcomes as transplant cell survival correlates directly with the extent of regeneration. Recent research shows that polymer-based hydrogels can offer protective transplant delivery solutions via an encapsulating matrix, resulting in better cell survival and distribution in neural injury sites [10]. It can be predicted that such protected delivery will become standard for future neural cell therapy on clinics. However, delivering transplant cells in polymer biomatrices raises several new considerations from a graft imaging perspective and needs development of imaging protocols tailored for this purpose. A few early studies show that pre-labelling neural transplant populations with NPs, then encapsulating these into hydrogels can potentially allow for MRI tracking of the intra-construct cells but more work is needed to develop optimized protocols for this evolving mode of cell delivery [11].

Many confounders still exist with NP use in neural transplant tracking, most notably the ability to achieve high initial graft labelling and to limit particle dilution with cell proliferation. Accumulation of signaling agents in neural immune cells can also generate ‘false positives’ impairing transplant cell detection [12]. These are not new ideas, in general, but do require attention for effective solutions to emerge. NP clearance is also likely to occur following immune infiltration of protective implanted biomaterials, although we are not aware of formal experimental evidence for this.

Complex versions of NP-based cell labelling strategies have been investigated and seem promising, particularly for magnetic localization of transplant populations. While stem cells can self-renew and replace cells lost to injury, another important ability they are believed to possess is ‘homing’ to sites of neural pathology, possibly due to ongoing inflammation or scarring in these areas [13]. Combining this ability with SPION labelling could potentially be used to increase stem cell localization, or direct migration to desired neuro-anatomical targets, using external magnetic fields. One such example of a complex therapy is SPION use with focused ultrasound (FUS) mediated blood brain barrier disruption, and magnetic Halbach arrays for cell localization/homing, which is suggested to improve cell localization even in deep brain sites [14]. Applying direct electrical currents may also improve the migratory activity of NP-labelled cells in brain tissue, but it is unclear whether this can be achieved for long distance or targeted migration [15].

Alongside the nanoengineering of transplant cells, there is a growing trend to use organic NPs for direct delivery of biomolecules such as neurotrophins (e.g. nerve growth factor), enzymes and drugs [16]. It is widely accepted that the complex pathology of neurological damage means that single therapeutic interventions are unlikely to be sufficient for regeneration and ‘combinatorial’ therapies are needed. The high functional versatility of NPs combined with the current move to encapsulate cells in implantable and injectable hydrogels means that designing advanced, multifunctional biomaterials for bespoke combination in regenerative neurology is realistic. For example, use of encapsulating hydrogels for protected stem cell delivery (for cell replacement) with simultaneous nanocarrier mediated delivery of neurotrophins to promote neuro-regeneration, immunomodulatory drugs to limit inflammatory processes and degradative enzymes to reduce glial scarring, could offer an advanced intervention. Dramatic progress in 3D printing of soft patterned materials raises the exciting possibility that topographic guidance cues can additionally be incorporated into such regenerative materials to guide neural cell outgrowth and could be a game-changer for combination therapy.

To realize this therapeutic vision, NPs with truly wide purpose, multifunctional capabilities need to be strategically designed and assembled. We propose a ‘compartmentalization’ approach needs to be considered for this. An inorganic core could provide labelling capability, leaving the polymer shell to load gene and drugs for combination therapy, however, a multi-layered shell may be essential to circumvent undesired interactions between nucleic acids and drugs. This is far from straight forward. Using block-copolymers seem to be a logical solution [17] but safety issues associated with these new, complex polymers needs to be carefully evaluated.

Neural tissue engineering has lagged significantly behind areas such as orthopedic tissue engineering, in bringing together research in biology and the physical sciences to generate innovative solutions for intractable medical conditions. Despite their extraordinary therapeutic potential, studies evaluating NP platforms for neural transplantation have tended to be sporadic. Future development urgently needs an appropriate combination of multidisciplinary expertise for design, development and testing of novel NP based neurotherapeutics.

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